Data Evaluation Report on the Toxicity of Ipconazole TG to Sheepshead Minnows (*Cyprinodon variegatus*), Early Life Cycle

PMRA Submission Number {.....} EPA MRID Number 50621302

Data Requirement:

PMRA Data Code	{.....}
EPA DP Barcode	449205
OECD Data Point	{.....}
EPA MRID	50621302
EPA Guideline	850.1400

Test Material: Ipconazole TG **Purity (%):** 96.7%
Common name: Ipconazole
Chemical name: IUPAC: (1RS,2SR,5RS;1RS,2SR,5SR)-2-(4-chlorobenzyl)-5-isopropyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
CAS name: 2-[(4-chlorophenyl)methyl]-5-(1-methylethyl)-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
CAS No.: 125225-28-7
Synonyms: None

Primary Reviewer: Rebecca L. Bryan **Signature:** Rebecca L. Bryan
Staff Scientist, CDM Smith/CSS-Dynamac JV **Date:** 3/11/2019

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Secondary Reviewer(s): Hannah Yingling **Date:** 04/28/2020
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Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
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Date Evaluation Completed: 31-03-2020

CITATION: Dinehart, S. 2018. Ipconazole: Early Life-Stage Toxicity Test with the Sheepshead Minnow, *Cyprinodon variegatus*, Under Flow-Through Conditions. Unpublished study performed by EAG Laboratories, Analytical Bio-Chemistry Laboratories, Inc. (a wholly owned subsidiary of EAG, Inc.), Columbia, Missouri. Laboratory Study No. 85889. Study sponsored by Kureha Corporation, Tokyo, Japan. Study initiated July 17, 2017 and completed June 27, 2018.

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

EXECUTIVE SUMMARY:

The 35-day chronic toxicity of Ipconazole to the early life-stage of the sheepshead minnow (*Cyprinodon variegatus*) was studied under flow-through conditions. Fertilized eggs/embryos (80/level, <24 hours old) were exposed to Ipconazole at nominal concentrations of 0 (negative and solvent controls), 0.075, 0.15, 0.30, 0.60, and 1.2 µg ai/L. The mean-measured concentrations <0.011 (<MQL, controls), 0.0676, 0.125, 0.260, 0.484, and 1.03 µg ai/L, respectively. The test system was maintained at 24.2 to 25.3°C and a pH of 7.7 to 8.5. No significant treatment-related effects were observed for hatching success, post-hatch survival, or growth at any treatment level compared to the control. There was a significant increase in the mean time to hatch by one or two days in treated groups, relative to the negative control ($p<0.05$); however, for mean time to hatch, the negative and solvent controls were significantly different ($p<0.05$). Given the lack of a concentration dependent response for time to hatch and lack of significant difference in mean time to hatch for treated groups relative to the solvent control, this increase in time to hatch relative to the negative control was not determined to be biologically significant. As a result, the reviewer determined the NOAEC and LOAEC to be 1.03 µg ai/L and >1.03 µg ai/L, respectively, for all endpoints based on mean-measured concentrations.

This study **is scientifically sound** and is classified as **acceptable**.

Results Synopsis

Test Organism Size/Age: Embryos, <24 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Endpoint(s) affected: mean time to hatch

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the U.S. EPA Ecological Effects Test Guideline OCSPP 850.1400: *Fish Early-Life Stage Toxicity Test* (1996) and OECD 210 - Fish Early-Life Stage Toxicity Test (2013). Deviations from OCSPP 850.1400 included:

1. Gentle aeration was used during the study starting on Day 28 until test termination.
2. The physiochemical properties of the test substance were not reported.
3. Several parameters of dilution water quality, including particulate matter, TOC, and organic chlorine, were not reported.
4. Dissolved oxygen concentration at day 0 was slightly above the recommended maximum (100% of saturation) in all replicates of the control treatment (values ranged from 7.4 to 7.5 mg/L or 103 to 104% of saturation). Dissolved oxygen concentration was below the recommended minimum (60% of saturation) in a single replicate of the 1.2 pg/L nominal treatment on study day 27 (measured value was 1.3 mg/L or 18% of saturation).
5. Light intensity was measured at five locations within the exposure system at test solution surface on study day 28 of the definitive test and ranged from 378 to 509 lux. These values were below the {protocol recommended minimum (540 lux).

This deviation does not impact the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality

statements were provided. This study was conducted in accordance with GLP Standards as published by the U.S. EPA (40 CFR Part 160), with the following exceptions: latest water and feed characterizations performed in 2015 and November 2017 were not performed in accordance to the stated GLP.

A. MATERIALS:

1. Test Material: Ipconazole

Description: Not reported

Lot No./Batch No.: 89010 (Batch No.)

Purity: 96.7% (containing 89.7% ipconazole cc and 7.0% ipconazole ct)

Stability of compound under test conditions: The reported mean measured concentrations were 81-90% of nominal. The coefficient of variance values ranged from 8 to 13%. A test substance stability trial was conducted from 29 to 30 January 2018 to investigate test substance stability in saltwater under static conditions. Concentrations of ipconazole, based on ipconazole cc and ipconazole ct analysis, were determined in samples using LC-MS/MS. Measured ipconazole concentration in nominal 1.2 µg/L solution was 85% of nominal at hour 0, and 73% of nominal or 86% of the hour 0 concentration following 19 hours of storage. These results demonstrated the test substance was stable in saltwater for at least 19 hours under static conditions and standard laboratory lighting.

Storage conditions of test chemicals: Room temperature in the dark

Physicochemical properties of Ipconazole.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Species: Sheepshead minnow (*Cyprinodon variegatus*)
EPA recommends rainbow trout (Oncorhynchus mykiss), bluegill sunfish (Lepomis macrochirus) or fathead minnow (Pimephales promelas) for freshwater, and sheepshead minnow (Cyprinodon variegatus) for estuarine/marine. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.

Age /embryonic stage at test initiation: Embryos, <24 hours old

EPA recommends fish embryo age at test initiation is as soon as possible after the eggs have been fertilized.

Method of collection of the fertilized eggs: Newly fertilized sheepshead minnows were collected. Based on a representative sample of embryos from the group used to initiate the test, the embryonic developmental stage of eggs at test initiation were predominantly early embryo stage.

Source: In-house laboratory culture

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 28-day flow through range-finding test was conducted with 20 embryos per replicate and two replicates per level, at nominal concentrations of (control), 0 (vehicle control, dimethylformamide), 0.038, 0.15, 0.30, 0.60, and 1.2 µg ai/L. Mean egg hatchability was 85, 93, 93, 83, 80, 85, and 83% in the 0 (control), 0 (vehicle control), 0.038, 0.15, 0.30, 0.60, and 1.2 µg ai/L groups, respectively. Mean fry survival at the end of the 28-day exposure (21 days post-hatch) was 100, 92, 92, 91, 100, 97, and 88% in the 0 (control), 0 (vehicle control), 0.038, 0.15, 0.30, 0.60, and 1.2 µg ai/L groups, respectively. Growth of surviving fry was assessed at test termination through individual length measurements and pooled replicate blotted wet weight measurements. Mean total length was 13.5, 13.3, 13.7, 13.5, 13.7, 13.3, and 10.8 mm in the 0 (control), 0 (vehicle control), 0.038, 0.15, 0.30, 0.60, and 1.2 µg ai/L groups, respectively. Mean individual blotted wet weight was calculated by dividing the total pooled replicate weight by the number of surviving fish. The mean individual blotted weights were 0.0337, 0.0348, 0.0356, 0.0348, 0.0362, 0.0312, and 0.0204 g in the 0 (control), 0 (vehicle control), 0.038, 0.15, 0.30, 0.60, and 1.2 µg ai/L groups, respectively. The results of this range-finding test were used to select the nominal concentrations for the definitive test. The highest nominal definitive test treatment (1.2 µg ai/L) was selected based on the 20 and 39% reduction in mean length and blotted wet weight, respectively, compared to the control.

b. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
<u>Parental acclimation, if any</u> Period: Conditions (same as test or not): Feeding (type, source, amount given, frequency): Health (any mortality observed):	 Continuous culture Not reported Not reported The adequate health of the sheepshead minnow brood stock was reported.	*EPA recommends embryos be immersed in the test solutions before cleavage of the blastodisc commences, or as close as possible after this stage.*
Number of fertilized eggs/embryos in each treatment at test initiation:	80 embryos/treatment level, divided into 20	*Each treatment should include a minimum of 60 embryos, divided equally*

Parameter	Details	Remarks *Criteria*
	embryos/chamber, and 4 replicate chambers/treatment	*between at least 2 replicate test chambers. OECD also recommends at least 60 eggs, divided between at least 2 replicates.*
Concentration of test material nominal: measured:	 0 (negative control), 0 (solvent control), 0.075, 0.15, 0.30, 0.60, and 1.2 µg ai/L <0.011 (<MQL, controls), 0.0676, 0.125, 0.260, 0.484, and 1.03 µg ai/L	Minimum Quantifiable Limit (MQL) for ipconazole cc = 0.0100 µg ai/L; and MQL for ipconazole ct = 0.00100 µg ai/L. Combined MQL is 0.011 µg ai/L. *A minimum of 5 concentrations, spaced by a constant factor not exceeding 3.2, and a control, all replicated, plus solvent control if appropriate should be used.* *- Toxicant concentration should be measured at least 5 times at regular intervals, and at least once per week for studies longer than one month.* *- Concentrations of test substance in solution should be within ± 20% of the mean measured values during study.* *- One concentration should adversely affect a life stage and one concentration should not affect any life stage.* *OECD also recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2 and concentrations be within ± 20% of the mean measured values.*
Solvent (type, percentage, if used)	Dimethylformamide (DMF); 25 µL/L	 *The solvent should not exceed 0.1 ml/L in a flow-through system. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, and ethanol.* *OECD recommends that the solvent not have an effect on survival nor produce any other adverse effects; concentration should not be greater than 0.1 ml/L.*
Number of replicates Negative control: Solvent control: Treatments:	 4 4 4/level	 *At least 2 replicates per test concentration and control.* *A solvent control should be used in conjunction with a solubilizing agent.*
Test condition static renewal/flow-through:		The accuracy of the diluter was verified before initiation. Additionally, mixing/flow-splitting cell delivery volumes were verified weekly during the test and splitter cup replicate delivery volumes were verified following test

Parameter	Details	Remarks *Criteria*
type of dilution system for flow through method: flow rate: renewal rate for static renewal:	Flow-through Proportional intermittent-flow diluter *ca.* 8.5 to 9.9 volume additions per day (see Reviewer's Comments) N/A	termination. The diluter was allowed to operate continuously at the in-life cycle rate for approximately nine days prior to initiation. Proper operation of the proportional diluter and all mechanical systems was verified twice each day. *Flow-through systems are generally recommended, including a system which continually dispenses and dilutes a stock solution of the test substance (e.g. metering pump, proportional diluter, or saturator system). EPA recommends that flow rate equal at least 5 test chamber volumes per 24 hours, and not vary by more than 10% throughout the test (OECD also recommends 5 test chamber volumes/24 hours). For flow through tests, biomass loading rate should not exceed 0.5 g/L/24 h and 5 g/L solution at any time. For static-renewal, EPA recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or retain organisms in vessels and change at least 2/3 test water.*
Aeration, if any	Gentle aeration was initiated in all test chambers on Day 28 and continued for the remainder of the test.	*Aeration is not recommended.*
Duration of the test	35 days: 7-day hatching period and 28-day post-hatch period	*EPA recommended test duration is 32 days (or 28 days post-hatch) for fathead minnow and sheepshead minnow; 30 days post-hatch for zebra fish and ricefish; 2 weeks after controls are free-feeding (or 60 days post-hatch) for rainbow trout; and 28 to 60 days for other species. OECD recommendations for test duration are species specific and range from 28-60 days.*
<u>Embryo cups, if used</u> type/material (glass/stainless steel): size: fill volume:	Glass cups with Nitex® screen bottoms 9 cm diameter Not reported	The incubation cups were suspended within each replicate aquarium. The cups were oscillated vertically in each aquarium by means of a rocker arm apparatus driven by a low rpm electric motor. *Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
<u>Test vessel</u> type/material: (glass/stainless steel)	Glass	The test chambers were scraped and siphoned clean on study days 5, 12, 19, 25, 31, and 33.

Parameter	Details	Remarks *Criteria*
size: fill volume:	22 cm wide x 38 cm long x 21.5 cm high, 12 cm depth 10 L	*EPA recommends any glass, stainless steel, or other chemically inert vessels can be used.*
Source of dilution water: <u>Quality of dilution water</u> Particulate matter: TOC: Un-ionized ammonia: Residual chlorine: Total organophosphorus pesticides: Total organochlorine pesticides + PCBs: Organic chlorine:	The dilution water was a laboratory saltwater prepared by adding a commercial sea salt mix (Crystal Sea Marinemix) to laboratory freshwater at a target salinity 20 ± 2‰. The laboratory freshwater consists of well water that was demineralized by reverse osmosis. Prior to use, the dilution water was aerated, heated, and passed through an ultraviolet sterilizer and a 1µm sediment filter. Not reported Not reported <0.10 mg/L (ammonia as N) <0.05 mg/L Not detected Not detected Not reported	The salt water was analyzed periodically for selected chemical parameters and potential contaminants and the November 2017 non-GLP analysis results were provided. *Any water with test species control survival meeting % hatching success and % post-hatch success in guideline is suitable as a test water. Maximum allowable concentrations for water quality parameters are provided in EPA's 850.1400 guideline (http://www.epa.gov/ocspp/pubs/frs/home/draftguidelines.htm). EPA also recommends testing for heavy metals (e.g. Cu, Pb, Zn, Hg, Cd, Ni), major anions and cations (e.g. Ca, Mg, Na, K, Cl, sulfate), and suspended solids. OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*

Parameter	Details	Remarks *Criteria*
Water quality during testing Hardness: pH: Dissolved oxygen: Temperature(s) (record all the temperatures used for different life stages): Salinity (for marine or estuarine species): Other measurements: Photoperiod: Interval of water quality measurements:	Not determined; Dilution water prepared with freshwater with a total hardness ranging from 130 to 160 mg $CaCO_3$/L. 7.7 to 8.5 1.3 to 7.5 mg/L (18-104% saturation; see Reviewer's Comments) Daily: 24.2 to 25.1°C Continuous: 25 ± 1.5°C 18.7 to 20.1‰ None 16-hour light/8-hour dark with 30-minute transition periods. Light intensity was 378 to 509 lux on Day 28 (see Reviewer's Comments). Temperature, pH, dissolved oxygen concentration, and salinity were measured in all control and treated replicates at test initiation, weekly throughout the test, and at termination of the definitive test. Additionally, temperature was continuously measured using a data logger and thermistor probe placed in a centrally located test chamber.	*EPA Recommendations:* *Dissolved Oxygen: 60 - 100% saturation. OECD recommends that DO concentration be between 60 - 90% saturation.* *Temperature for fathead minnow, zebra fish and sheepshead minnow: 25±2°C; rainbow trout: 10-12±2°C, depending on life stage; and ricefish: 23-24±1-2°C, depending on life stage. Temperature should not deviate by more than ±1.5 °C between test chambers or between successive days during test.* *Salinity for sheepshead minnow: 15-30 ‰ ±2%; salinity for silverside: 20 ‰.* *Recommended photoperiod: 12-16 hours, depending on species.* *EPA recommends DO, salinity (if relevant), and temperature be measured weekly, and pH and hardness be measured at the beginning and end of the test. Temperature should preferably be monitored continuously in at least one test vessel.* *OECD also recommends at a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test.*
Post-hatch details when the post-hatch period began: number of hatched eggs (alevins)/ treatment released to the test chamber: on what day, the alevins were released from the incubation cups to the test chamber:	 Day 7 All live fry Day 12	Mean hatching success was 95% for the negative control and 98% for the solvent control. *Hatching success in each control should be ≥ 66% for rainbow trout and fathead minnow; >75% for sheepshead minnow; and >66 or >80% for other species as shown.* *Post-Hatch success in each control should be ≥ 70% in rainbow trout, fathead minnow and zebra fish; ≥ 80% in ricefish and sheepshead minnow; and ≥ 60 to ≥ 80% for other species as shown.*

Parameter	Details	Remarks *Criteria*
Post-hatch Feeding start date: type/source of feed: amount given: frequency of feeding:	 Day 7 Live brine shrimp nauplii (*Artemia salina*) and starting on study Day 27 additional standard commercial fish food was provided. Food was provided three times daily. Starting with the 2nd feeding on study Day 13 (Day 6 post-hatch) and for the reminder of the test, feeding volume was adjusted to account for mortality so that feeding rate was approximately equivalent across all chambers. Fish were not fed during the 24 hours prior to study termination.	*EPA's 850.1400 guidelines for feeding and handling recommendations for brood and test animals of recommended species and other species are at (https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0154-0033).*
Stability of chemical in the test system	The mean measured concentrations were 81-90% of nominal with coefficient of variance ranging from 8 to 13%.	
Recovery of chemical: Frequency of measurement: LOD: MQL:	81 to 90% of nominal Days 0, 7, 14, 19, 28, and 35 Not reported ipconazole cc = 0.0100 µg ai/L; ipconazole ct = 0.00100 µg ai/L	Recoveries based on mean measured concentrations. Test solutions were analyzed for the concentration of ipconazole, based on ipconazole cc and ipconazole ct analysis, using a liquid chromatography system with tandem mass spectrometry (LC-MS/MS). The combined Minimum Quantifiable Limit (MQL) is 0.011 µg ai/L.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	 Not reported At test initiation	Based on a representative sample of embryos from the group used to initiate the test.

Parameter	Details	Remarks *Criteria*
Other parameters, if any	The maximum calculated dynamic biomass loading at test termination was 0.0215 g/L/day, which satisfied the biomass loading requirement stated in the study protocol (i.e., 0.5 g/L/day).	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	- Embryonic development - Hatchability - Time to hatch - Survival (Day 28 post-hatch) - Growth (length and wet weight) - Behavioral or physical changes (including abnormalities)	*Recommended parameters measured include:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and juveniles;* *- Time to swim-up (if appropriate);* *- Measurement of growth (length and weight); dry weight (24 hours at 60°C) is preferred to wet weight;* *- Incidence and description of morphological abnormalities and behavioral effects;* *- Observations of other effects or clinical signs.*
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g. alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects:	 Daily Daily Daily N/A Day 35 (Day 28 post-hatch) At test initiation Daily	*Observations on hatching and survival should be made at least once daily; length and weight at end of test; and morphological abnormalities and behavioral effects at adequate intervals, depending on duration of test.*
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY: Hatching success averaged 94-98% in all treatment groups (including the controls). No significant effects in hatching success were observed. The NOAEC and LOAEC values for hatching success were 1.03 and >1.03 µg ai/L, respectively, based on mean-measured concentrations.

No treatment-related effects in post-hatch survival were observed and averaged 93-99% in all groups (including the controls). The NOAEC and LOAEC values for post-hatch survival were 1.03 and >1.03 µg ai/L, respectively, based on mean-measured concentrations.

Table 3: Effect of Ipconazole on Egg Hatching and Survival at Different Life Stages of Sheepshead Minnow (*Cyprinodon variegatus*).[a]

Mean-measured (and Nominal) Concentrations (µg ai/L)	Eggs hatched/embryo viability			Mean Time to Hatch (Days)	Juvenile-survival on day 28 post-hatch	
	No. of eggs at study initiation	Hatch/embryo viability				
		No.	%		No.	% Survival
Negative Control (<MQL) [b]	80	76	95	5	71	93
Solvent Control (<MQL) [b]	80	78	98	7	77	99
0.0676 (0.075)	80	77	96	7	74	96
0.125 (0.15)	80	77	96	7	76	99
0.260 (0.30)	80	75	94	6	73	97
0.484 (0.60)	80	76	95	7	74	97
1.03 (1.2)	80	77	96	7	74	96
NOAEC	1.03 µg ai/L			1.03 µg ai/L	1.03 µg ai/L	
LOAEC	>1.03 µg ai/L			>1.03 µg ai/L	>1.03 µg ai/L	

a Data were obtained from Tables 4 and 6 on pages 34-35 and 37 of the study report. The reviewer calculated the total number of hatched embryos and the total number of surviving juveniles from replicate data.
b MQL = 0.011 µg ai/L

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Time to hatch: No treatment-related effect on the time to hatch was observed (started on Days 4 to 6). The mean times to hatch completion were 9, 8, 8, 8, and 9 days in the mean-measured 0.0676, 0.125, 0.260, 0.484, and 1.03 µg ai/L groups, respectively, compared to 7-8 days in the controls. The estimated NOAEC and LOAEC for time to hatch completion were 1.03 and >1.03 µg ai/L, respectively, based on mean-measured concentrations.

Clinical signs of toxicity: Based on a representative sample of embryos from the group used to initiate the test, the embryonic developmental stage of eggs at test initiation ranged from blastoderm with germ ring to early embryo stage.

No treatment related clinical signs of toxicity were reported. The estimated NOAEC and LOAEC for clinical signs of toxicity were 1.03 and >1.03 µg ai/L, respectively, based on mean-measured concentrations.

Growth: Total lengths averaged 18.9, 19.0, 19.2, 18.8, and 18.5 mm in the mean-measured 0.0676, 0.125, 0.260, 0.484, and 1.03 µg ai/L groups, respectively, compared to 18.8-18.9 mm in the controls. The wet

weights averaged 0.0849, 0.0906, 0.0938, 0.0877, and 0.0842 g in the mean-measured 0.0676, 0.125, 0.260, 0.484, and 1.03 µg ai/L groups, respectively, compared to 0.0879 g in the negative control and 0.0850 g in the solvent control. The NOAEC and LOAEC for growth were 1.03 and >1.03 µg ai/L, respectively, based on mean-measured concentrations.

Table 4: Effect of Ipconazole on Growth of Juvenile Sheepshead Minnow (*Cyprinodon variegatus*). [a]

Mean-measured (and Nominal) Concentrations (µg ai/L)	Swim-up (days)			Growth-length (mm ± SD)	Growth-wet weight (g ± SD)
	day x1	day x2	day xn		
Negative Control (<MQL) [b]	N/A	N/A	N/A	18.9±0.47	0.0879±0.00879
Solvent Control (<MQL) [b]	N/A	N/A	N/A	18.8±0.20	0.0850±0.00393
0.0676 (0.075)	N/A	N/A	N/A	18.9±0.27	0.0849±0.00477
0.125 (0.15)	N/A	N/A	N/A	19.0±0.28	0.0906±0.00407
0.260 (0.30)	N/A	N/A	N/A	19.2±0.23	0.0938±0.00535
0.484 (0.60)	N/A	N/A	N/A	18.8±0.21	0.0877±0.00718
1.03 (1.2)	N/A	N/A	N/A	18.5±0.38	0.0842±0.00503
NOAEC	N/A	N/A	N/A	1.03 µg ai/L	1.03 µg ai/L
LOAEC	N/A	N/A	N/A	>1.03 µg ai/L	>1.03 µg ai/L

a Data were obtained from Table 9 on page 40 of the study report.
b MQL = 0.011 µg ai/L

C. REPORTED STATISTICS:

Data that were statistically analyzed included egg hatchability, time to hatch completion, post-hatch fry survival, and growth at exposure termination (total length and blotted wet weight). No statistical analyses were performed on the time to hatch start data. Prior to comparisons of the treatment groups to the control, the negative control and vehicle control were compared to determine if differences between control treatments were statistically significant. For mean time to hatch, there was a statistically significant difference between the negative control and vehicle control. The determination of no-observed adverse effect concentration (NOAEC) for time to hatch were made against both controls. For all other endpoints, there no statistical difference between the controls, and comparisons for determination of a NOAEC were made against the negative control. Additional investigational comparisons were made against the vehicle control for time to hatch completion.

All data were checked for normality using Shapiro-Wilk's test (p=0.01) and for homogeneity of variance using Levene's test (p=0.01). The overall survival met the assumptions of normality and homogeneity. The time to hatch, time to hatch completion, egg hatchability, and fry survival data were non-normal and non-monotonic. The NOAEC for time to hatch start and time to hatch completion was estimated using a one-way analysis of variance (ANOVA), and a one-tailed Dunnett's test, and Williams' test with the alternate hypothesis being the parameter mean was increased compared to the control mean. The NOAEC for egg hatchability (i.e., proportion hatched), fry survival (proportion surviving at 28-days post-hatch), and overall survival (proportion of initial embryos surviving at 28-days post-hatch) was estimated using an ANOVA procedure and a one-tailed Dunnett's test, and Williams' test with the alternate hypothesis being the parameter mean was reduced in comparison to the control mean.

The EC_{10} and EC_{20} values could not be determined for time to hatch start, time to hatch, and time to hatch completion because no biologically relevant responses were detected. No estimate of EC_{10} and EC_{20} values were determined for post-hatch fry survival, overall survival, length, or weight because reductions were <10% in the treatments compared to the control.

All statistical tests were performed using mean-measured concentrations and SAS software (version 9.3). Inferences of statistical significance were based on a p=0.05 significance level unless otherwise noted, and all comparisons were made against the control group.

Egg hatchability
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Days to Hatch Start
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Days to Hatch Completion
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Time to Hatch
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Post-hatch survival
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Overall survival
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Total length
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Wet weight
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Endpoint(s) affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer analyzed hatching success, mean time to hatch, post-hatch survival, and growth (wet weight and length) using CETIS statistical software version 1.9.5.3 with database backend settings implemented by EFED on 7/25/2017.

Data were then tested for normality using the Shapiro-Wilk's test ($\alpha = 0.01$) and for homogeneity of variance using Bartlett's or Levene's test ($\alpha = 0.01$). All endpoints met these assumptions and were therefore analyzed using analysis of variance followed by Dunnett's or Williams' multiple comparison tests. All analyses were conducted $\alpha = 0.05$ unless specified otherwise, and all toxicity values are based on the mean-measured concentrations.

Hatching success
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Time to Hatch*
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Post-hatch survival
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Total length
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Wet weight
NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

**Significant effects at each treatment level for time to hatch did not appear to dose-dependent, but all were significantly delayed a day or two, relative to the negative control mean time to hatch (day 5); relative to the solvent control, mean time to hatch was not significantly different in any treatment.*

Endpoint(s) affected: None that were biologically significant (*above)

E. STUDY DEFICIENCIES:

There were no deviations and/or deficiencies from OCSPP guidance affecting the scientific soundness or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with those reported by the study author for all endpoints. The study author's analysis observed significant increases in time to hatch in the test substance treatments compared to the negative control; however the negative and solvent controls also were significantly different for mean time to hatch. The study author deemed these increases compared to the negative control not to be biologically relevant due to the lack of a dose-dependent response and the reviewer agreed.

The validity criteria that were met included control hatching success of >70% (91% during study), control post-hatch survival of >75% (96% during study), and water temperature did not differ by more than ± 1.5°C between test chambers or between successive days at any time during the test.

Light intensity ranged from 378 to 509 lux at test solution surface on study day 28 of the definitive test. While lighting intensity was below the protocol recommended minimum (540 lux), all light readings were 401 lux or greater with a single exception (378 lux). This departure from recommended light intensity had no effect on the study as demonstrated by acceptable hatch and fry survival in the controls.

Test solution dissolved oxygen concentration was generally maintained between 60% and 100% saturation (ranged from 1.3 to 7.5 mg/L; 18 to 104% saturation). The maximum dissolved oxygen concentration was slightly above the recommended maximum of 100% saturation at Day 0 in all control test chambers (7.4 to 7.5 mg/L or 103 to 104% saturation). This had no effect on the test as indicated by acceptable hatch and fry survival in the control treatment. The minimum dissolved oxygen concentration (1.3 mg/L or 18% of saturation

in one 1.03 µg/L replicate on Day 27) resulted from a 19-hour test solution delivery line obstruction. If this single value is eliminated, the lowest dissolved oxygen reading was 4.4 mg/L (61% saturation). The obstruction was cleared and dissolved oxygen concentration had improved to 4.6 mg/L (64% saturation) later the same day. This event had no adverse effect on study integrity because the duration of reduced dissolved oxygen concentration in this chamber was short relative to study duration. Also, data from the test solution stability trial indicates exposure concentrations were likely maintained in the 1.03 µg/L replicate while test solution flow was reduced.

For Days 0 to 27, the diluter cycle rate was set to approximately 7.1 cycles/hour, which was sufficient to provide approximately 8.5 volume additions to each test chamber over a 24-hour period. In an attempt to increase test solution dissolved oxygen concentration, the diluter cycle rate was increased on Day 27 to achieve a target of approximately 8.1 cycles/hour from Days 28 to 35, which was sufficient to provide approximately 9.9 volume additions to each test chamber over a 24-hour period.

The definitive study was conducted from November 3, 2017 to December 8, 2017.

G. CONCLUSIONS:

This study **is scientifically sound** and is classified as **acceptable**. No significant treatment-related effects were observed for hatching success, post-hatch survival, or growth at any treatment level compared to the control. Statistically significant effects on time to hatch were observed at all treatment levels compared to the negative control ($p<0.05$). However, these statistically significant delays of one to two days did not appear to be dose-dependent and were consistent with the delay in solvent control. Therefore, the slight delay in time to hatch was deemed biologically insignificant, resulting in a NOAEC of 1.03 µg ai/L (the highest treatment concentration) and a LOAEC of >1.03 µg ai/L.

NOAEC: 1.03 µg ai/L
LOAEC: >1.03 µg ai/L

Endpoint(s) affected: None that were biologically significant

III. REFERENCES:

None; other than standard guidelines and methodologies

OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Analytical Bio-Chemistry Laboratories, Inc.**

Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Sample ID:	00-5460-0594	**Code:**	50621302	**Project:**	Fungicide
Sample Date:	03 Nov-17	**Material:**	Ipconazole	**Source:**	Kureha Corporation
Receipt Date:		**CAS (PC):**		**Station:**	
Sample Age:	n/a	**Client:**	CDM Smith - E. Krupka		

PC Code 125618 MRID 50621302 mean-measured concentrations

SIngle Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
19-2547-0444	Hatching Success	Equal Variance t Two-Sample Test	0.5370	Solvent Blank passed hatching success	1
10-8455-0274	Mean Length	Equal Variance t Two-Sample Test	0.6963	Solvent Blank passed mean length	1
09-4577-4480	Mean Wet Weight	Equal Variance t Two-Sample Test	0.5764	Solvent Blank passed mean wet weight	1

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	TU	PMSD	S
12-3188-2692	Hatching Success	Dunnett Multiple Comparison Test		1.03	>1.03	n/a		10.1%	1
06-7297-5477	Hatching Success	Williams Multiple Comparison Test		1.03	>1.03	n/a		7.85%	1
15-3760-7000	Mean Length	Dunnett Multiple Comparison Test		1.03	>1.03	n/a		2.85%	1
19-7947-6018	Mean Length	Williams Multiple Comparison Test		1.03	>1.03	n/a		2.21%	1
11-4110-3471	Mean Wet Weight	Dunnett Multiple Comparison Test		1.03	>1.03	n/a		11.7%	1
11-3854-2271	Mean Wet Weight	Williams Multiple Comparison Test		1.03	>1.03	n/a		9.1%	1
14-0855-1649	Time to Hatch	Dunnett Multiple Comparison Test	✓	<0.0676	0.0676	n/a		14.5%	1
13-9962-4700	Time to Hatch	Williams Multiple Comparison Test	✓	<0.0676	0.0676	n/a		11.2%	1

OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Analytical Bio-Chemistry Laboratories, Inc.**

Hatching Success Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	0.9750	0.9291	1.0000	0.9500	1.0000	0.0144	0.0289	2.96%	0.00%
0	N	4	0.9500	0.8375	1.0000	0.8500	1.0000	0.0354	0.0707	7.44%	2.56%
0.0676		4	0.9625	0.8863	1.0000	0.9000	1.0000	0.0239	0.0479	4.97%	1.28%
0.125		4	0.9625	0.8863	1.0000	0.9000	1.0000	0.0239	0.0479	4.97%	1.28%
0.26		4	0.9375	0.8182	1.0000	0.8500	1.0000	0.0375	0.0750	8.00%	3.85%
0.484		4	0.9500	0.8581	1.0000	0.9000	1.0000	0.0289	0.0577	6.08%	2.56%
1.03		4	0.9625	0.9227	1.0000	0.9500	1.0000	0.0125	0.0250	2.60%	1.28%

Mean Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	18.77	18.5	19.05	18.6	19	0.08539	0.1708	0.91%	0.00%
0	N	4	18.88	18.15	19.6	18.3	19.4	0.2287	0.4573	2.42%	-0.53%
0.0676		4	18.88	18.46	19.29	18.5	19.1	0.1315	0.263	1.39%	-0.53%
0.125		4	19	18.53	19.47	18.7	19.3	0.1472	0.2944	1.55%	-1.20%
0.26		4	19.18	18.82	19.53	18.9	19.4	0.1109	0.2217	1.16%	-2.13%
0.484		4	18.8	18.51	19.09	18.6	19	0.09129	0.1826	0.97%	-0.13%
1.03		4	18.55	17.93	19.17	18	18.9	0.1936	0.3873	2.09%	1.20%

Mean Wet Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	0.08498	0.07872	0.09123	0.0815	0.0904	0.001964	0.003928	4.62%	0.00%
0	N	4	0.08778	0.07404	0.1015	0.0797	0.1	0.004315	0.00863	9.83%	-3.30%
0.0676		4	0.0849	0.07731	0.09249	0.0782	0.0887	0.002386	0.004773	5.62%	0.09%
0.125		4	0.09062	0.08417	0.09708	0.0869	0.0951	0.002027	0.004054	4.47%	-6.65%
0.26		4	0.0938	0.0853	0.1023	0.0868	0.0988	0.002672	0.005344	5.70%	-10.39%
0.484		4	0.08772	0.07628	0.09917	0.0776	0.0929	0.003595	0.00719	8.20%	-3.24%
1.03		4	0.0842	0.07617	0.09223	0.0778	0.09	0.002523	0.005046	5.99%	0.91%

Time to Hatch Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	7	7	7	7	7	0	0	0.00%	0.00%
0	N	4	5	5	5	5	5	0	0	0.00%	28.57%
0.0676		4	6.5	5.581	7.419	6	7	0.2887	0.5774	8.88%	7.14%
0.125		4	6.75	5.954	7.546	6	7	0.25	0.5	7.41%	3.57%
0.26		4	6.25	5.454	7.046	6	7	0.25	0.5	8.00%	10.71%
0.484		4	6.75	5.954	7.546	6	7	0.25	0.5	7.41%	3.57%
1.03		4	7	7	7	7	7	0	0	0.00%	0.00%

OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **Analytical Bio-Chemistry Laboratories, Inc.**

Hatching Success Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	1.0000	0.9500	0.9500	1.0000
0	N	1.0000	1.0000	0.8500	0.9500
0.0676		1.0000	0.9500	0.9000	1.0000
0.125		1.0000	0.9500	1.0000	0.9000
0.26		1.0000	1.0000	0.9000	0.8500
0.484		1.0000	1.0000	0.9000	0.9000
1.03		1.0000	0.9500	0.9500	0.9500

Mean Length Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	19	18.7	18.8	18.6
0	N	19.4	18.3	19	18.8
0.0676		19	18.9	19.1	18.5
0.125		19.2	18.8	18.7	19.3
0.26		18.9	19.1	19.4	19.3
0.484		18.7	18.6	19	18.9
1.03		18	18.6	18.7	18.9

Mean Wet Weight Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	0.0904	0.0815	0.0852	0.0828
0	N	0.1	0.0797	0.0854	0.086
0.0676		0.0879	0.0848	0.0887	0.0782
0.125		0.0951	0.0869	0.0875	0.093
0.26		0.0926	0.0868	0.097	0.0988
0.484		0.0876	0.0776	0.0928	0.0929
1.03		0.0778	0.0836	0.0854	0.09

Time to Hatch Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	7	7	7	7
0	N	5	5	5	5
0.0676		7	6	6	7
0.125		6	7	7	7
0.26		6	6	7	6
0.484		7	6	7	7
1.03		7	7	7	7

OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	12-3188-2692	**Endpoint:**	Hatching Success	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:10	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	1.03	>1.03	n/a		10.13%

Dunnett Multiple Comparison Test

Control	vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.0676	-0.3128	2.407	0.096	6	CDF	0.9090	Non-Significant Effect
		0.125	-0.3128	2.407	0.096	6	CDF	0.9090	Non-Significant Effect
		0.26	0.3128	2.407	0.096	6	CDF	0.7253	Non-Significant Effect
		0.484	0	2.407	0.096	6	CDF	0.8333	Non-Significant Effect
		1.03	-0.3128	2.407	0.096	6	CDF	0.9090	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0020833	0.0004167	5	0.1304	0.9834	Non-Significant Effect
Error	0.0575	0.0031944	18			
Total	0.0595833		23			

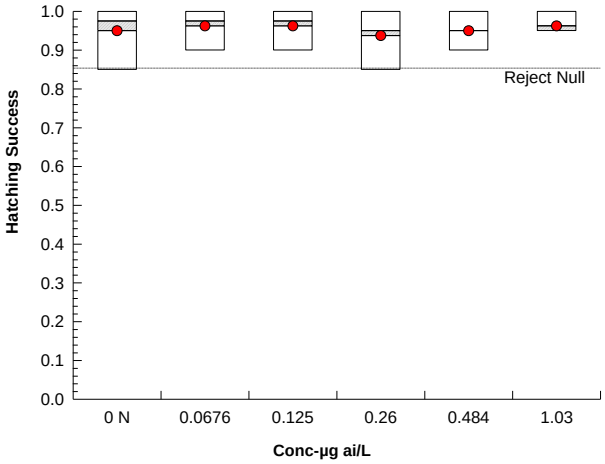
ANOVA Assumptions Tests

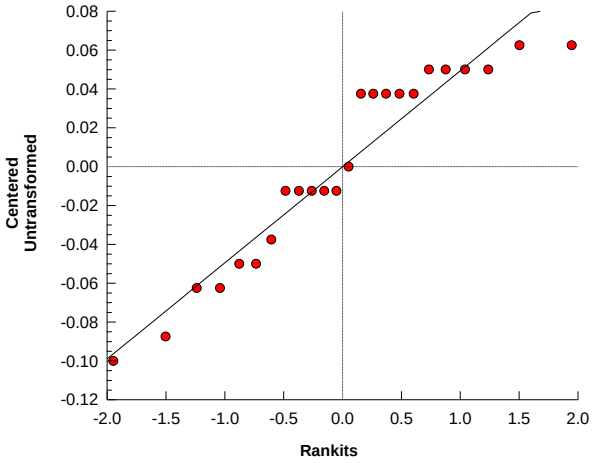
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	3.291	15.09	0.6552	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9058	0.884	0.0287	Normal Distribution

Hatching Success Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.9500	0.8375	1.0000	0.9750	0.8500	1.0000	0.0354	7.44%	0.00%
0.0676		4	0.9625	0.8863	1.0000	0.9750	0.9000	1.0000	0.0239	4.97%	-1.32%
0.125		4	0.9625	0.8863	1.0000	0.9750	0.9000	1.0000	0.0239	4.97%	-1.32%
0.26		4	0.9375	0.8182	1.0000	0.9500	0.8500	1.0000	0.0375	8.00%	1.32%
0.484		4	0.9500	0.8581	1.0000	0.9500	0.9000	1.0000	0.0289	6.08%	0.00%
1.03		4	0.9625	0.9227	1.0000	0.9500	0.9500	1.0000	0.0125	2.60%	-1.32%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	06-7297-5477	**Endpoint:**	Hatching Success	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:10	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	1.03	>1.03	n/a		7.85%

Williams Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.0676	-0.3128	1.734	0.069	6	CDF	>0.05	Non-Significant Effect
	0.125	-0.3128	1.818	0.073	6	CDF	>0.05	Non-Significant Effect
	0.26	0.3128	1.845	0.074	6	CDF	>0.05	Non-Significant Effect
	0.484	0.1564	1.859	0.074	6	CDF	>0.05	Non-Significant Effect
	1.03	0	1.867	0.075	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0020833	0.0004167	5	0.1304	0.9834	Non-Significant Effect
Error	0.0575	0.0031944	18			
Total	0.0595833		23			

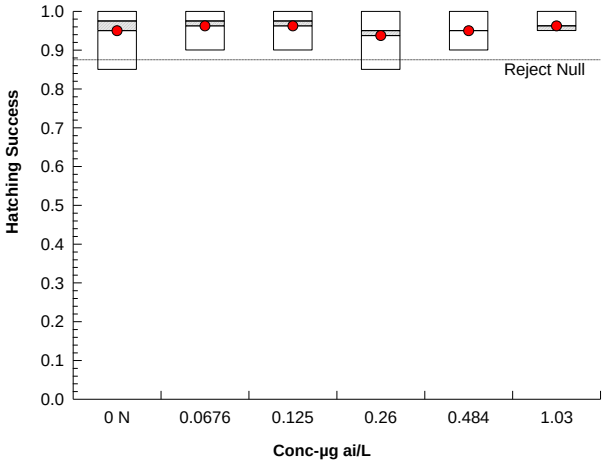
ANOVA Assumptions Tests

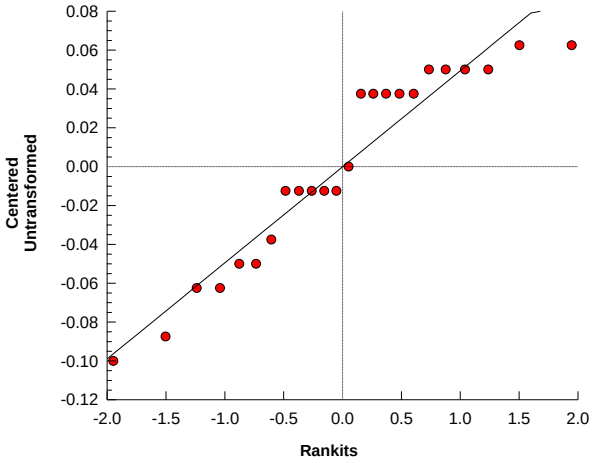
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	3.291	15.09	0.6552	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9058	0.884	0.0287	Normal Distribution

Hatching Success Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.9500	0.8375	1.0000	0.9750	0.8500	1.0000	0.0354	7.44%	0.00%
0.0676		4	0.9625	0.8863	1.0000	0.9750	0.9000	1.0000	0.0239	4.97%	-1.32%
0.125		4	0.9625	0.8863	1.0000	0.9750	0.9000	1.0000	0.0239	4.97%	-1.32%
0.26		4	0.9375	0.8182	1.0000	0.9500	0.8500	1.0000	0.0375	8.00%	1.32%
0.484		4	0.9500	0.8581	1.0000	0.9500	0.9000	1.0000	0.0289	6.08%	0.00%
1.03		4	0.9625	0.9227	1.0000	0.9500	0.9500	1.0000	0.0125	2.60%	-1.32%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	19-2547-0444	**Endpoint:**	Hatching Success	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:11	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed hatching success	9.84%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.6547	2.447	0.093	6	CDF	0.5370	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.00125	0.00125	1	0.4286	0.5370	Non-Significant Effect
Error	0.0175	0.0029167	6			
Total	0.01875		7			

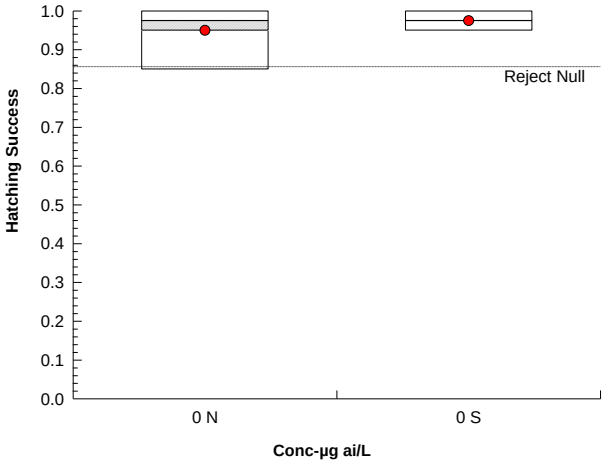
ANOVA Assumptions Tests

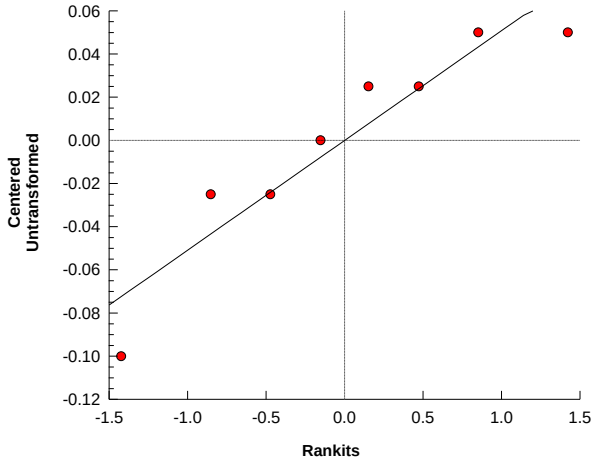
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	6	47.47	0.1753	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.8879	0.6451	0.2235	Normal Distribution

Hatching Success Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	0.9750	0.9291	1.0000	0.9750	0.9500	1.0000	0.0144	2.96%	0.00%
0	N	4	0.9500	0.8375	1.0000	0.9750	0.8500	1.0000	0.0354	7.44%	2.56%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	15-3760-7000	**Endpoint:**	Mean Length	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:10	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	1.03	>1.03	n/a		2.85%

Dunnett Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.0676	0	2.407	0.537	6	CDF	0.8333	Non-Significant Effect
	0.125	-0.5602	2.407	0.537	6	CDF	0.9475	Non-Significant Effect
	0.26	-1.344	2.407	0.537	6	CDF	0.9934	Non-Significant Effect
	0.484	0.3361	2.407	0.537	6	CDF	0.7160	Non-Significant Effect
	1.03	1.456	2.407	0.537	6	CDF	0.2422	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.867083	0.173417	5	1.741	0.1762	Non-Significant Effect
Error	1.7925	0.0995833	18			
Total	2.65958		23			

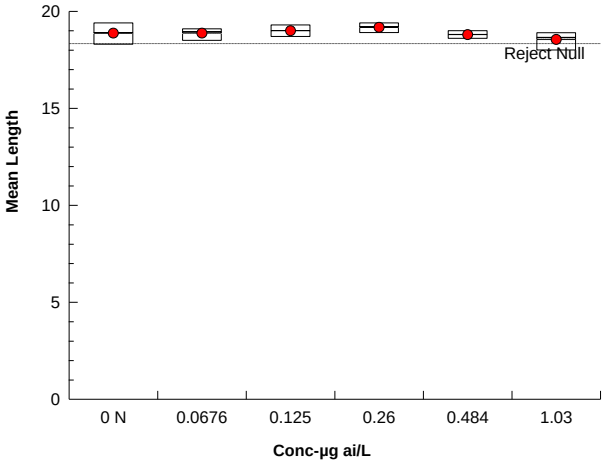
ANOVA Assumptions Tests

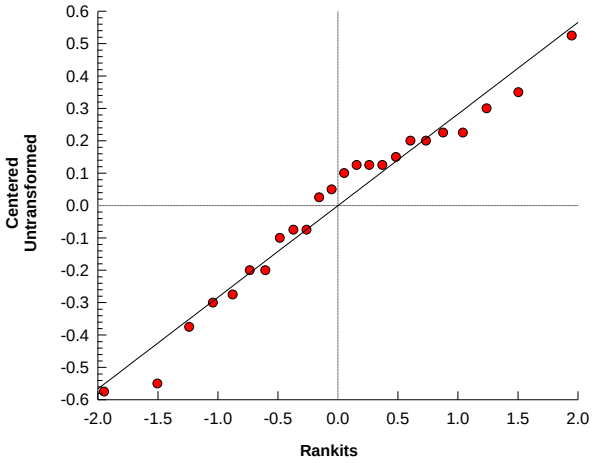
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	3.059	15.09	0.6909	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9631	0.884	0.5041	Normal Distribution

Mean Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	18.87	18.15	19.6	18.9	18.3	19.4	0.2287	2.42%	0.00%
0.0676		4	18.88	18.46	19.29	18.95	18.5	19.1	0.1315	1.39%	0.00%
0.125		4	19	18.53	19.47	19	18.7	19.3	0.1472	1.55%	-0.66%
0.26		4	19.17	18.82	19.53	19.2	18.9	19.4	0.1109	1.16%	-1.59%
0.484		4	18.8	18.51	19.09	18.8	18.6	19	0.0913	0.97%	0.40%
1.03		4	18.55	17.93	19.17	18.65	18	18.9	0.1937	2.09%	1.72%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	19-7947-6018	**Endpoint:**	Mean Length	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:10	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	1.03	>1.03	n/a		2.21%

Williams Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.0676	0	1.734	0.387	6	CDF	>0.05	Non-Significant Effect
	0.125	-0.2801	1.818	0.406	6	CDF	>0.05	Non-Significant Effect
	0.26	-0.6349	1.845	0.412	6	CDF	>0.05	Non-Significant Effect
	0.484	0.3361	1.859	0.415	6	CDF	>0.05	Non-Significant Effect
	1.03	1.456	1.867	0.417	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.867083	0.173417	5	1.741	0.1762	Non-Significant Effect
Error	1.7925	0.0995833	18			
Total	2.65958		23			

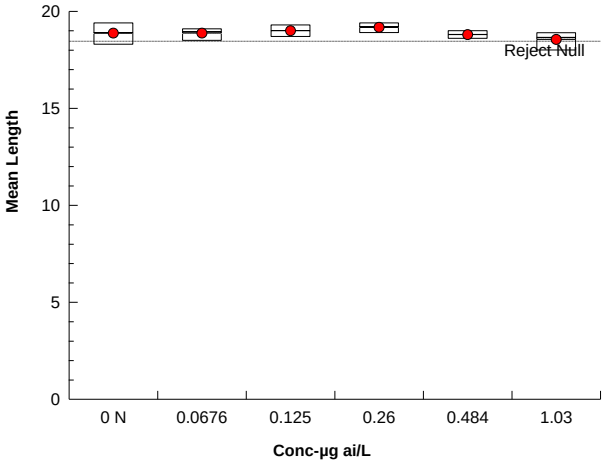
ANOVA Assumptions Tests

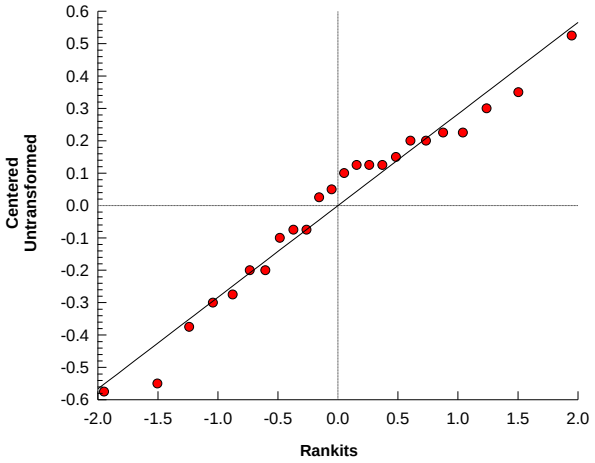
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	3.059	15.09	0.6909	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9631	0.884	0.5041	Normal Distribution

Mean Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	18.87	18.15	19.6	18.9	18.3	19.4	0.2287	2.42%	0.00%
0.0676		4	18.88	18.46	19.29	18.95	18.5	19.1	0.1315	1.39%	0.00%
0.125		4	19	18.53	19.47	19	18.7	19.3	0.1472	1.55%	-0.66%
0.26		4	19.17	18.82	19.53	19.2	18.9	19.4	0.1109	1.16%	-1.59%
0.484		4	18.8	18.51	19.09	18.8	18.6	19	0.0913	0.97%	0.40%
1.03		4	18.55	17.93	19.17	18.65	18	18.9	0.1937	2.09%	1.72%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	10-8455-0274	**Endpoint:**	Mean Length	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:11	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed mean length	3.16%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.4097	2.447	0.597	6	CDF	0.6963	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02	0.02	1	0.1678	0.6963	Non-Significant Effect
Error	0.715	0.119167	6			
Total	0.735		7			

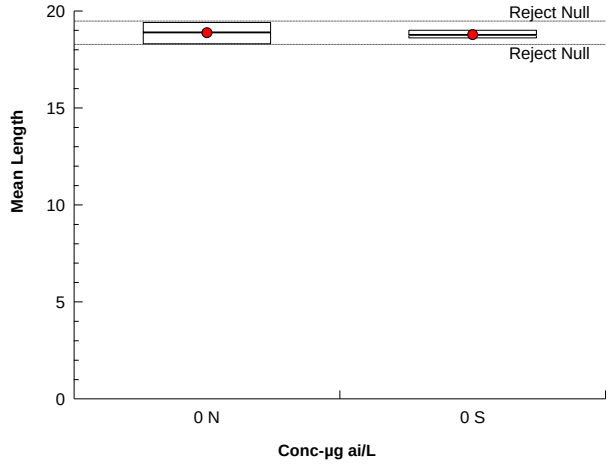
ANOVA Assumptions Tests

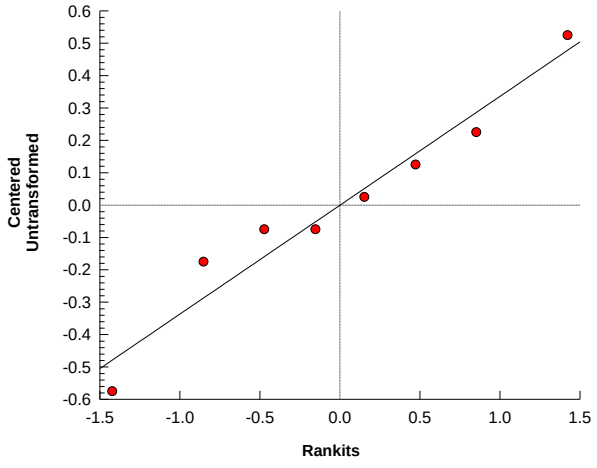
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	7.171	47.47	0.1399	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9699	0.6451	0.8973	Normal Distribution

Mean Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	18.78	18.5	19.05	18.75	18.6	19	0.0854	0.91%	0.00%
0	N	4	18.87	18.15	19.6	18.9	18.3	19.4	0.2287	2.42%	-0.53%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	11-4110-3471	**Endpoint:**	Mean Wet Weight	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:10	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	1.03	>1.03	n/a		11.73%

Dunnett Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.0676	0.6724	2.407	0.010	6	CDF	0.5706	Non-Significant Effect
	0.125	-0.6665	2.407	0.010	6	CDF	0.9592	Non-Significant Effect
	0.26	-1.409	2.407	0.010	6	CDF	0.9946	Non-Significant Effect
	0.484	0.01169	2.407	0.010	6	CDF	0.8299	Non-Significant Effect
	1.03	0.8361	2.407	0.010	6	CDF	0.4957	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0002581	5.163E-05	5	1.412	0.2672	Non-Significant Effect
Error	0.0006582	3.657E-05	18			
Total	0.0009164		23			

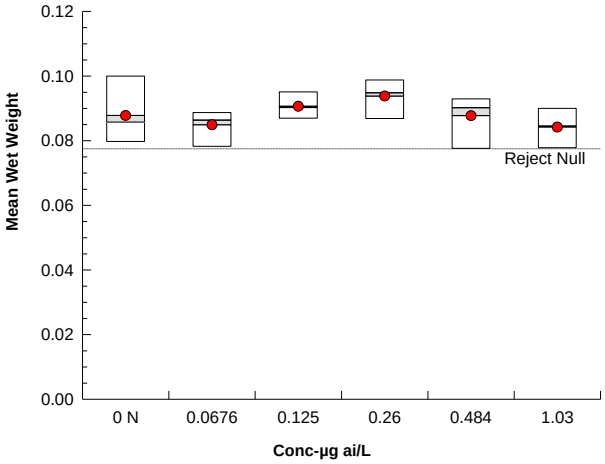
ANOVA Assumptions Tests

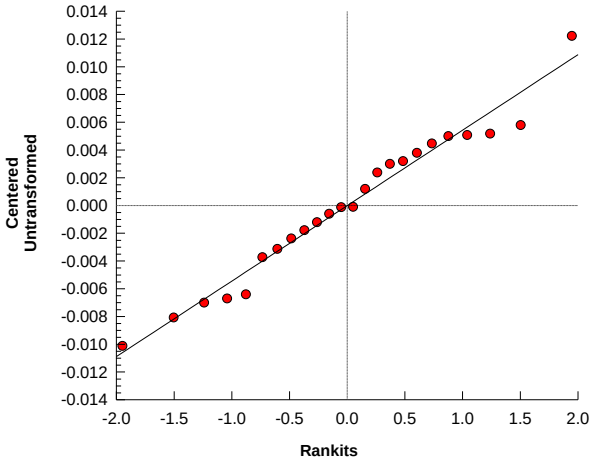
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.19	15.09	0.8223	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9728	0.884	0.7366	Normal Distribution

Mean Wet Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.08778	0.07404	0.1015	0.0857	0.0797	0.1	0.004315	9.83%	0.00%
0.0676		4	0.0849	0.07731	0.09249	0.08635	0.0782	0.0887	0.002386	5.62%	3.28%
0.125		4	0.09063	0.08417	0.09708	0.09025	0.0869	0.0951	0.002027	4.47%	-3.25%
0.26		4	0.0938	0.0853	0.1023	0.0948	0.0868	0.0988	0.002672	5.70%	-6.86%
0.484		4	0.08773	0.07628	0.09917	0.0902	0.0776	0.0929	0.003595	8.20%	0.06%
1.03		4	0.0842	0.07617	0.09223	0.0845	0.0778	0.09	0.002523	5.99%	4.07%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	11-3854-2271	**Endpoint:**	Mean Wet Weight	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:10	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	1.03	>1.03	n/a		9.10%

Williams Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.0676	0.6724	1.734	0.007	6	CDF	>0.05	Non-Significant Effect
	0.125	0.002923	1.818	0.008	6	CDF	>0.05	Non-Significant Effect
	0.26	-0.4677	1.845	0.008	6	CDF	>0.05	Non-Significant Effect
	0.484	0.01169	1.859	0.008	6	CDF	>0.05	Non-Significant Effect
	1.03	0.8361	1.867	0.008	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0002581	5.163E-05	5	1.412	0.2672	Non-Significant Effect
Error	0.0006582	3.657E-05	18			
Total	0.0009164		23			

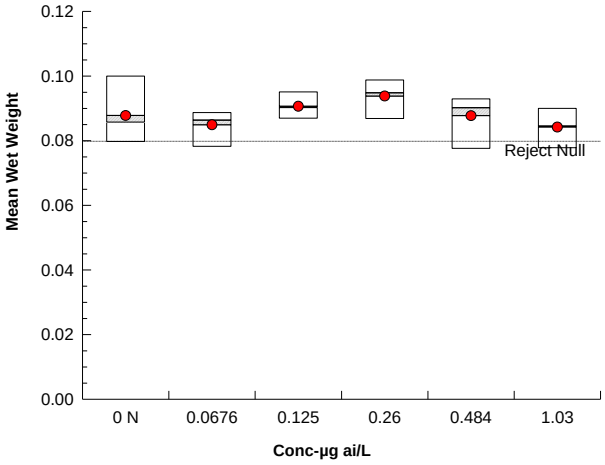
ANOVA Assumptions Tests

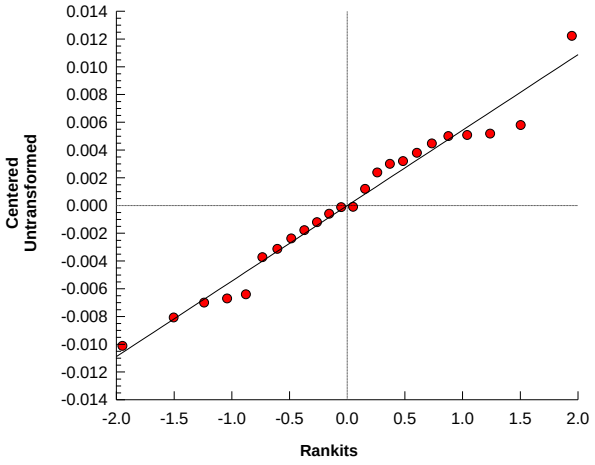
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.19	15.09	0.8223	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9728	0.884	0.7366	Normal Distribution

Mean Wet Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.08778	0.07404	0.1015	0.0857	0.0797	0.1	0.004315	9.83%	0.00%
0.0676		4	0.0849	0.07731	0.09249	0.08635	0.0782	0.0887	0.002386	5.62%	3.28%
0.125		4	0.09063	0.08417	0.09708	0.09025	0.0869	0.0951	0.002027	4.47%	-3.25%
0.26		4	0.0938	0.0853	0.1023	0.0948	0.0868	0.0988	0.002672	5.70%	-6.86%
0.484		4	0.08773	0.07628	0.09917	0.0902	0.0776	0.0929	0.003595	8.20%	0.06%
1.03		4	0.0842	0.07617	0.09223	0.0845	0.0778	0.09	0.002523	5.99%	4.07%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	09-4577-4480	**Endpoint:**	Mean Wet Weight	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:11	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed mean wet weight	13.22%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.5906	2.447	0.012	6	CDF	0.5764	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.568E-05	1.568E-05	1	0.3488	0.5764	Non-Significant Effect
Error	0.0002697	4.496E-05	6			
Total	0.0002854		7			

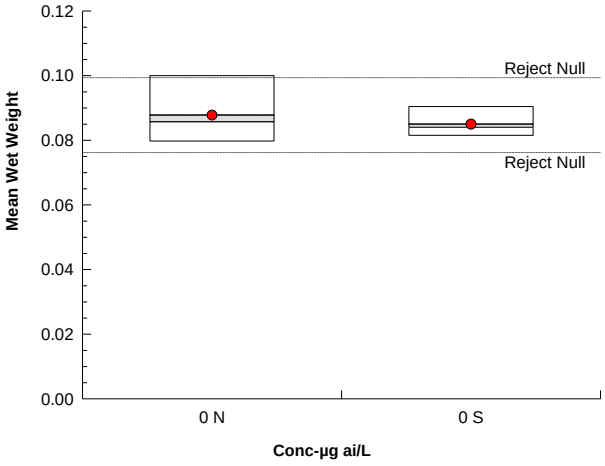
ANOVA Assumptions Tests

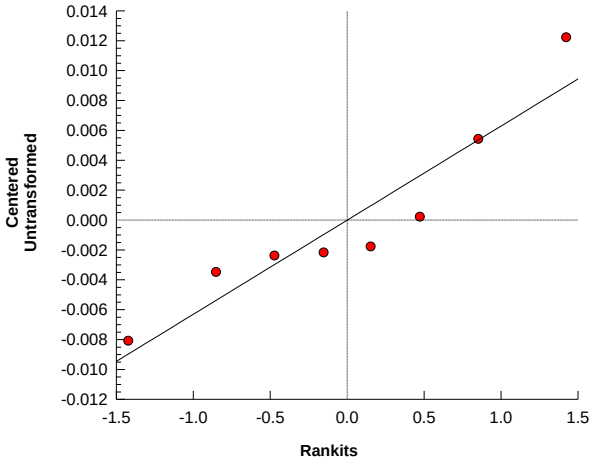
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	4.827	47.47	0.2285	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.8994	0.6451	0.2855	Normal Distribution

Mean Wet Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	0.08498	0.07872	0.09123	0.084	0.0815	0.0904	0.001964	4.62%	0.00%
0	N	4	0.08778	0.07404	0.1015	0.0857	0.0797	0.1	0.004315	9.83%	-3.30%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	14-0855-1649	**Endpoint:**	Time to Hatch	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:10	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	<0.0676	0.0676	n/a		14.46%

Dunnett Multiple Comparison Test

Control vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	0.0676*	4.992	2.407	0.723	6	CDF	2.4E-04	Significant Effect
	0.125*	5.824	2.407	0.723	6	CDF	6.3E-05	Significant Effect
	0.26*	4.16	2.407	0.723	6	CDF	0.0013	Significant Effect
	0.484*	5.824	2.407	0.723	6	CDF	6.3E-05	Significant Effect
	1.03*	6.656	2.407	0.723	6	CDF	3.3E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	10.375	2.075	5	11.49	4.3E-05	Significant Effect
Error	3.25	0.180556	18			
Total	13.625		23			

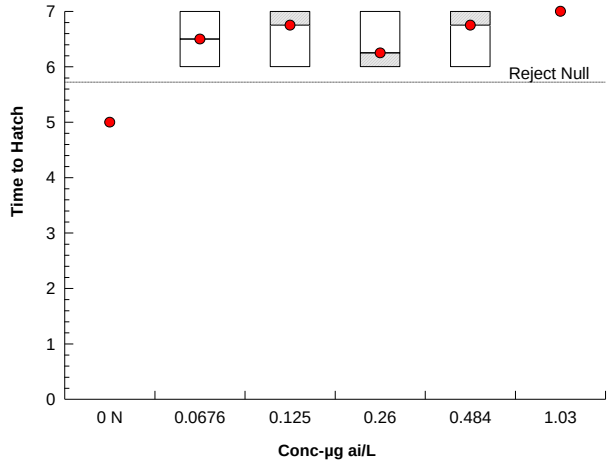
ANOVA Assumptions Tests

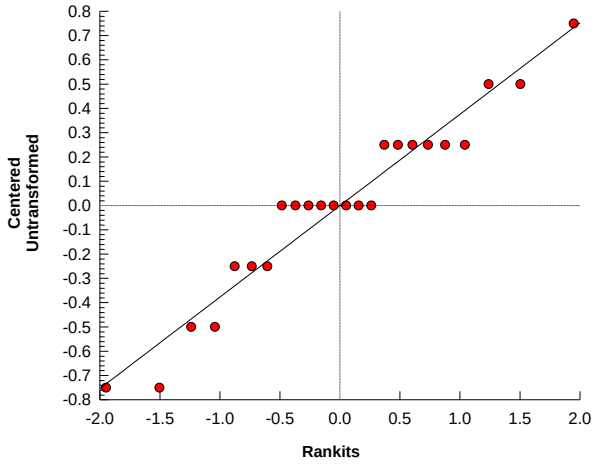
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	5.933	4.248	0.0021	Unequal Variances
	Mod Levene Equality of Variance Test	1.133	4.248	0.3786	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9428	0.884	0.1880	Normal Distribution

Time to Hatch Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	5	5	5	5	5	5	0	0.00%	0.00%
0.0676		4	6.5	5.581	7.419	6.5	6	7	0.2887	8.88%	-30.00%
0.125		4	6.75	5.954	7.546	7	6	7	0.25	7.41%	-35.00%
0.26		4	6.25	5.454	7.046	6	6	7	0.25	8.00%	-25.00%
0.484		4	6.75	5.954	7.546	7	6	7	0.25	7.41%	-35.00%
1.03		4	7	7	7	7	7	7	0	0.00%	-40.00%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	13-9962-4700	**Endpoint:**	Time to Hatch	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:10	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	<0.0676	0.0676	n/a		11.22%

Williams Multiple Comparison Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.0676*	4.992	1.734	0.521	6	CDF	<0.05	Significant Effect
		0.125*	5.408	1.818	0.546	6	CDF	<0.05	Significant Effect
		0.26*	4.16	1.845	0.554	6	CDF	<0.05	Significant Effect
		0.484*	4.992	1.859	0.559	6	CDF	<0.05	Significant Effect
		1.03*	5.492	1.867	0.561	6	CDF	<0.05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	10.375	2.075	5	11.49	4.3E-05	Significant Effect
Error	3.25	0.180556	18			
Total	13.625		23			

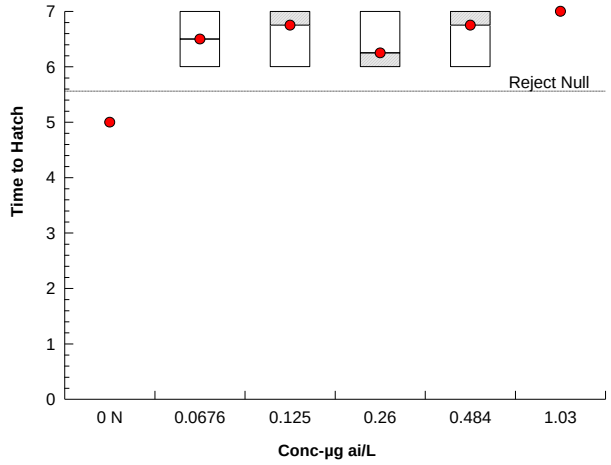
ANOVA Assumptions Tests

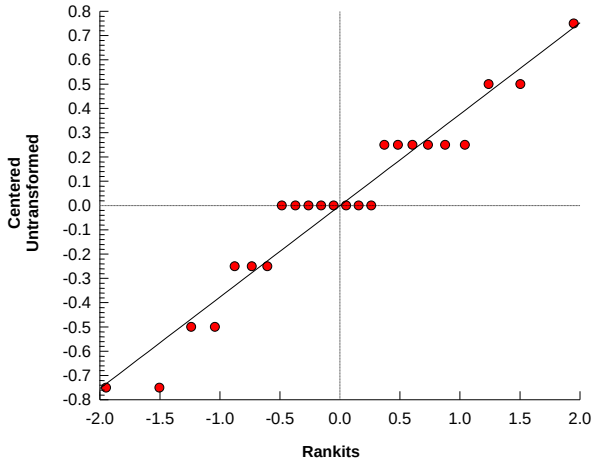
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	5.933	4.248	0.0021	Unequal Variances
	Mod Levene Equality of Variance Test	1.133	4.248	0.3786	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9428	0.884	0.1880	Normal Distribution

Time to Hatch Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	5	5	5	5	5	5	0	0.00%	0.00%
0.0676		4	6.5	5.581	7.419	6.5	6	7	0.2887	8.88%	-30.00%
0.125		4	6.75	5.954	7.546	7	6	7	0.25	7.41%	-35.00%
0.26		4	6.25	5.454	7.046	6	6	7	0.25	8.00%	-25.00%
0.484		4	6.75	5.954	7.546	7	6	7	0.25	7.41%	-35.00%
1.03		4	7	7	7	7	7	7	0	0.00%	-40.00%

Graphics





OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) — **Analytical Bio-Chemistry Laboratories, Inc.**

Analysis ID:	01-0485-4310	**Endpoint:**	Time to Hatch	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Apr-19 14:11	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	03-2763-3898	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	03 Nov-17	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage (	**Diluent:**	Laboratory Seawater
Ending Date:	08 Dec-17	**Species:**	Cyprinodon variegatus	**Brine:**	
Test Length:	35d 0h	**Taxon:**	Actinopterygii	**Source:**	Analytical Bio-chemistry lab **Age:**

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	8	8	1	65540	<1.0E-37	Significant Effect
Error	0	0	6			
Total	8		7			

Time to Hatch Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	7	7	7	7	7	7	0	0.00%	0.00%
0	N	4	5	5	5	5	5	5	0	0.00%	28.57%

Graphics